

WHAT I CLAIM AS MY INVENTION IS:

1. A method for the expression of a heterologous polypeptide by a host cell said method comprising:
 - 5 a) introducing into a host cell a chimeric nucleic acid sequence comprising:
 - 1) a first nucleic acid sequence capable of regulating the transcription in said host cell of
 - 2) a second nucleic acid sequence, wherein said second
10 sequence encodes a fusion polypeptide and comprises (i) a nucleic acid sequence encoding a sufficient portion of an oil body protein gene to provide targeting of the fusion polypeptide to a lipid phase linked in reading frame to (ii) a nucleic acid sequence encoding the heterologous polypeptide; and
 - 3) a third nucleic acid sequence encoding a termination
15 region functional in the host cell; and
 - b) growing said host cell to produce the fusion polypeptide.
2. The method according to claim 1 further including separating the recombinant fusion polypeptide from cellular host cell components by selective
20 partitioning into a lipid phase.
3. The method according to claim 2 wherein said selective partitioning comprises centrifugation, floatation or size exclusion.
- 25 4. The method according to claim 1 further including separating the recombinant fusion polypeptide from cellular host components by selective partitioning into a lipid phase comprising oil bodies.
5. The method according to claim 4 wherein said recombinant fusion
30 polypeptide is separated by addition of oil body components and reconstitution of the oil bodies.

6. The method according to claim 2 further comprising releasing the heterologous polypeptide from the fusion polypeptide associated with the lipid phase, said method comprising:

- c) including in said second nucleic acid sequence (2) between said
5 nucleic acid sequence (i) encoding the oil body protein and the nucleic acid sequence (ii) encoding the heterologous polypeptide, a linker nucleic acid sequence (iii) encoding an amino acid sequence that is specifically cleavable by enzymatic or chemical means; and
- d) contacting the lipid phase with said enzymatic or chemical means
10 such that said heterologous polypeptide is released from the fusion polypeptide.

7. The method according to claim 6 wherein said linker nucleic acid sequence encodes an amino acid sequence that is recognizable by the proteolytic action of an enzyme selected from the group consisting of thrombin, factor Xa,
15 collagenase, chymosin, clostrypain and viral protease.

8. The method according to claim 6 wherein said enzymatic means comprises an enzyme that is immobilized.

20 9. The method according to claim 8 wherein said enzyme is immobilized by attachment to an oil body protein that is associated with an oil body.

10. The method according to claim 1 wherein said recombinant
25 polypeptide is an enzyme.

11. The method according to claim 10 wherein said recombinant polypeptide is an enzyme that retains its enzymatic properties while part of the fusion polypeptide is associated with the oil body.

12. A method for the production and /release of a heterologous polypeptide from a fusion polypeptide associated with a plant oil body fraction during seed germination and plant seedling growth, said method comprising:
- a) introducing into a plant cell a first chimeric nucleic acid sequence
- 5 comprising:
- 1) a first nucleic acid sequence capable of regulating the transcription in said plant cell of
 - 2) a second nucleic acid sequence wherein said nucleic acid second sequence encodes a fusion polypeptide and comprises (i) a nucleic acid
 - 10 sequence encoding a sufficient portion of an oil body protein gene to provide targeting of the fusion polypeptide to an oil body, linked in reading frame to (ii) a nucleic acid sequence encoding the heterologous polypeptide and (iii) a linker nucleic acid sequence encoding an amino acid sequence that is specifically cleavable by enzymatic means wherein said linker nucleic acid sequence (iii) is
 - 15 located between said nucleic acid sequence (i) encoding the oil body protein and said nucleic acid sequence (ii) encoding the recombinant polypeptide; and
 - 3) a third nucleic acid sequence encoding a termination region;
- b) sequentially or concomitantly introducing into the genome of said
- 20 plant a second chimeric nucleic acid sequence comprising:
- 1) a first nucleic acid sequence capable of regulating the transcription specifically during seed germination and seed growth of
 - 2) a second nucleic acid sequence encoding a specific enzyme that is capable of cleaving the linker nucleic acid sequence of said first
 - 25 chimeric nucleic acid sequence; and
 - 3) a third nucleic acid sequence encoding a termination region;
- c) regenerating a plant from said plant cell and growing said plant to produce seed whereby said fusion polypeptide is expressed and associated with
- 30 oil bodies and
- d) allowing said seed to germinate wherein said enzyme in said second chimeric nucleic acid sequence is expressed and cleaves the heterologous

polypeptide from the fusion polypeptide associated with the oil bodies during seed germination and early seedling growth.

13. A method for producing an altered seed meal by producing a
5 heterologous polypeptide in association with a plant seed oil body fraction, said method comprising:
- a) introducing into a plant cell a chimeric nucleic acid sequence comprising:
- 1) a first nucleic acid sequence capable of regulating the
10 transcription in said plant cell of
- 2) a second nucleic acid sequence wherein said second sequence encodes a fusion polypeptide and comprises (i) a nucleic acid sequence encoding a sufficient portion of an oil body protein gene to provide targeting of the fusion polypeptide to an oil body, linked in reading frame to (ii) a nucleic
15 acid sequence encoding the heterologous polypeptide and
- 3) a third nucleic acid sequence encoding a termination region;
- b) regenerating a plant from said plant cell and growing said plant to produce seed whereby said heterologous polypeptide is expressed and
20 associated with oil bodies; and
- c) crushing said seed and preparing an altered seed meal.

14. A method of preparing an enzyme in a host cell in association with an oil body and releasing said enzyme from the oil body, said method
25 comprising:
- a) transforming a host cell with a chimeric nucleic acid sequence comprising:
- 1) a first nucleic acid sequence capable of regulating the transcription of
- 30 2) a second nucleic acid sequence, wherein said second sequence encodes a fusion polypeptide and comprises (i) a nucleic acid sequence encoding a sufficient portion of an oil body protein gene to provide targeting of

- the fusion polypeptide to an oil body; (ii) a nucleic acid sequence encoding an enzyme and (iii) a linker nucleic acid sequence located between said nucleic acid sequence (i) encoding the oil body and said nucleic acid sequence (ii) encoding the enzyme and encoding an amino acid sequence that is cleavable by the enzyme encoded by the nucleic acid sequence (ii); and
- 5 3) a third nucleic acid sequence encoding a termination region functional in said host cell
- b) growing the host cell to produce the fusion polypeptide under conditions such that enzyme is not active;
- 10 c) recovering the oil bodies containing the fusion polypeptide; and
- d) altering the environment of the oil bodies such that the enzyme is activated and cleaves itself from the fusion polypeptide.

15. The method according to claim 14 wherein said enzyme is

15 activated by lowering the pH or altering the temperature of the oil body environment.

16. A method for the expression of a heterologous polypeptide by a host cell in association with an oil body and separating said heterologous
- 20 polypeptide from the oil body, said method comprising:
- a) transforming a first host cell with a first chimeric nucleic sequence comprising:
- 1) a first nucleic acid sequence capable of regulating the transcription in said host cell of
- 25 2) a second nucleic acid sequence, wherein said second sequence encodes a first fusion polypeptide and comprises (i) a nucleic acid sequence encoding a sufficient portion of an oil body protein gene to provide targeting of the first fusion polypeptide to a lipid phase linked in reading frame to (ii) a nucleic acid sequence encoding the heterologous polypeptide; and (iii) a
- 30 linker nucleic acid sequence encoding an amino acid sequence that is specifically cleavable by enzymatic means wherein said linker nucleic acid sequence (iii) is

located between said (i) nucleic acid sequence encoding the oil body protein and said (ii) nucleic acid sequence encoding the heterologous polypeptide; and

3) a third nucleic acid sequence encoding a termination region functional in the host cell; and

5 b) transforming a second host cell with a second chimeric nucleic acid sequence comprising:

1) a first nucleic acid sequence capable of regulating the transcription specifically during seed germination and seed growth of

2) a second nucleic acid sequence wherein said second
10 sequence encodes a second fusion polypeptide and comprises (i) a nucleic acid sequence encoding a sufficient portion of an oil body protein gene to provide targeting of the second fusion polypeptide to a lipid phase linked in reading frame to do a nucleic acid sequence, encoding a specific enzyme that is capable of cleaving the linker nucleic acid sequence of said first chimeric nucleic acid
15 sequence; and

3) a third nucleic acid sequence encoding a termination region;

c) growing said first host cell under conditions such that the first fusion polypeptide is expressed and associated with the oil bodies to produce a
20 first oil body fraction containing the first fusion polypeptide;

d) growing said second host cell under conditions such that the second fusion polypeptide is expressed and associated with the oil bodies to product a second oil body fraction containing the second fusion polypeptide;

e) contacting the first oil body fraction of step (c) with the second oil
25 body fraction of step (d) under conditions such that the enzyme portion of the second fusion polypeptide cleaves the heterologous polypeptide from the first fusion polypeptide.

17. The method according to claim 1 wherein said heterologous
30 polypeptide is selected from the group consisting of antibodies, glycanases, hormones, proteases, protease inhibitors and seed storage proteins.

18. The method according to claim 1 wherein said heterologous polypeptide is selected from the group consisting of a thrombin inhibitor, hirudin, an interleuken, chymosin, cystatin, xylanase, carp growth hormone, zein, an antibody and a collagenase.
- 5 19. The method according to claim 1 wherein said host cell is a plant cell.
20. The method according to claim 19 wherein said plant is
10 dicotyledonous.
21. The method according to claim 19 wherein said plant is monocotyledonous.
- 15 22. The method according to claim 19 wherein said plant is from the family *Brassicaceae*, *Compositae*, *Euphorbiaceae*, *Leguminosae*, *Linaceae*, *Malvaceae*, *Umbelliferae* or *Graminae*.
- 20 23. The method according to claim 21 wherein said plant is from the species *Brassica napus* (canola), *Helianthus annuus* (sunflower), *Carthamus tinctorius* (safflower), *Glycine max* (soybean), *Ricinus communis* (castor bean), *Linum usitatissimum* (flax), *Gossypium hirsutum* (cotton), *Coriandrum sativum* (coriander) or *Zea mays* (corn).
- 25 24. The method according to claim 1 wherein said host cell is a bacterial cell.
25. The method according to claim 1 wherein said host cell is a yeast cell.
- 30 26. The method according to claim 25 wherein said yeast cell is *Saccharomyces cerevisiae*.

27. The method according to claim 1 wherein said host cell is an insect or animal cell.

5 28. A method according to claim 1 wherein said oil body protein gene is from a plant.

29. A method according to claim 28 wherein said oil body protein gene is an oleosin or a caleosin.

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30. A method for the expression of a heterologous polypeptide by a host cell said method comprising:

a) generating by homologous recombination into a host cell a chimeric nucleic acid sequence comprising:

15 1) a first nucleic acid sequence capable of regulating transcription in said host cell

2) a second nucleic acid sequence, wherein said second sequence encodes a fusion polypeptide and comprises (i) a nucleic acid sequence encoding a sufficient amount of an oil body protein gene to provide targeting of
20 the fusion polypeptide to a lipid phase, linked in reading frame to (ii) a nucleic acid sequence encosing the heterologous polypeptide; and

3) a third nucleic acid sequence encoding a termination region functional in the host cell; and

b) growing said hot cell to produce the heterologous polypeptide.

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31. A chimeric nucleic acid sequence, capable of being expressed in association with an oil body of a host cell comprising:

1) a first nucleic acid sequence capable of regulating the transcription in said host cell of

30 2) a second nucleic acid sequence, wherein said second sequence encodes a fusion polypeptide and comprises (i) a nucleic acid sequence encoding a sufficient portion of an oil body protein gene to provide targeting of

the fusion polypeptide to a lipid phase linked in reading frame to (ii) a nucleic acid sequence encoding the heterologous polypeptide; and

3) a third nucleic acid sequence encoding a termination region functional in the host cell.

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32. The chimeric nucleic acid sequence according to claim 31 wherein said nucleic acid sequence (ii) encodes an enzyme.

33. The chimeric nucleic acid sequence according to claim 31 further including (iii) a linker nucleic acid sequence encoding an amino acid sequence that is specifically cleavable by enzymatic means wherein said linker nucleic acid sequence (iii) is located between said (i) nucleic acid sequence encoding the oil body protein and said (ii) nucleic acid sequence encoding the heterologous polypeptide.

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34. The chimeric nucleic acid according to claim 33 wherein said linker nucleic acid sequence (iii) encodes a cleavage site for an enzyme selected from the group consisting of thrombin, factor Xa, collagenase chymosin and viral protease.

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35. A chimeric nucleic acid sequence according to claim 33 wherein said oil body protein gene is from a plant.

36. A chimeric nucleic acid sequence according to claim 35 wherein said oil body protein gene is an oleosin or a caleosin.

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37. An expression cassette comprising a chimeric nucleic acid sequence according to claim 31.

38. A plant transformed with a chimeric nucleic acid sequence according to claim 31.

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39. A plant cell culture containing a chimeric nucleic acid sequence according to claim 31.
40. A plant seed containing a chimeric nucleic acid sequence according
5 to claim 31.
41. A fusion polypeptide encoded for by a chimeric nucleic acid sequence comprising (i) a nucleic acid sequence encoding a sufficient portion of an oil body protein gene to provide targeting of the fusion polypeptide to a lipid
10 phase and (ii) a nucleic acid sequence encoding a heterologous polypeptide.